APPENDIX

METHODS

Study design

This was a prospective, international, randomised, double blind, parallel group study performed in Europe and South Africa between April 2003 and October 2004. The study protocol was prepared in accordance with the Declaration of Helsinki and ethical approval was obtained for each centre. Written, informed consent was obtained from each participant.

Inclusion and exclusion criteria

The main inclusion criteria for the study were women with uncomplicated pelvic inflammatory disease (PID), as confirmed by the absence of pelvic or tubo-ovarian abscess on transabdominal/transvaginal pelvic ultrasound and/or laparoscopy (within 2 days before or 1 day after the start of treatment). Diagnosis of PID was based on the presence of all of the following symptoms: pelvic discomfort, direct lower abdominal tenderness with or without rebound tenderness and adnexal/cervical motion tenderness on bimanual vaginal examination. In addition, the women also had to have at least one of the following signs: raised temperature ($\geqslant$37.5°C), erythrocyte sedimentation rate $>$15 mm in the first hour, C reactive protein value above the upper limit of the normal range, white blood cell count $>10\,500 \times 10^6$/l, laparoscopic evidence of PID, signs suggestive of cervical infection (for example, mucopurulent cervical discharge), or untreated, documented gonococcal or chlamydial cervicitis within the previous 14 days. The women underwent testing to exclude pregnancy and had to agree to the use of barrier contraception during the study. All participants had to have had cervical/endocervical culture and polymerase chain reaction (PCR) testing for microbial pathogens within 48 hours before the start of the study.

In addition to contraindications to study drugs, women were excluded if they required surgery within the next 24 hours or had a history of uterine or pelvic or abdominal surgery within the past 30 days, or received previous treatment with systemic antibiotic therapy in the last 7 days.

Study assessments

Before treatment, participants were questioned about their symptoms and past history of PID and general health status. Within 48 hours before the start of treatment, baseline demographic and medical history data were collected, a physical examination was performed and clinical, ultrasound, laparoscopic (in some subjects), microbiological and laboratory assessments were carried out. Pelvic tenderness/pain was assessed at the abdominal and pelvic examination using a modified McCormack score (table A).[1] Pain was also assessed by the subject herself using a visual analogue scale where 0 mm = ''no pain'' and 100 mm = ''worst possible pain.'' If the woman was using an intrauterine device (IUD), the device was removed, at the latest within 24 hours after the initiation of treatment.

Microbiological examination of cervical/endocervical specimens was performed for all participants. If an endometrial biopsy was performed or if the women underwent culdocentesis or laparoscopy, specimens were obtained for culture and the isolation of aerobic and anaerobic bacteria. The material obtained for culture was forwarded to the local microbiology department for routine microscopy, Gram staining and bacterial culture processing. In addition, samples were subjected to a nucleic acid amplification test using PCR for *Neisseria gonorrhoeae, Mycoplasma genitalium* and *Chlamydia trachomatis*. This was also done retrospectively from pre-treatment samples for *M hominis*. All tests were performed at a central laboratory. Cobas Amplicor PCR tests were used for the detection of *N gonorrhoeae* and *C trachomatis*. Concerning genital mycoplasmas—that is, *M genitalium* and *M hominis*, the central laboratory used a real time PCR (Lightcycler Roche) method that was validated by Roche.

The women were instructed about the need for treatment compliance and their sexual partners were referred to an sexually transmitted disease (STD) clinic for evaluation and treatment. It was requested that they abstain from coitus until the completion of treatment, or else use barrier contraception.

The women were examined again during treatment (days 4–7) for clinical status and any adverse events. After treatment, most of the baseline physical and clinical examinations and assessments were repeated at a test of cure (TOC) visit on day 5–24 post-therapy and at an additional follow up visit on day 28–42 post-therapy in successfully treated subjects and in those evaluated as ''indeterminate'' at the TOC visit who had not been administered an alternative antibiotic therapy. Bacteriological assessments were performed on patients who had had a pretreatment organism isolated. Safety data were recorded. Whenever possible, the same investigator at each site carried out all assessment visits for individual subjects.

Drug administration

Participants were randomly assigned in a 1:1 ratio to the two treatment groups. During the 14 day treatment period, patients in the moxifloxacin group received oral moxifloxacin, 400 mg once daily, while patients in the comparator group received oral ofloxacin, 400 mg twice daily combined with oral metronidazole, 500 mg twice daily. Treatment could be administered either on an inpatient or outpatient basis. Blinding was achieved by dispensing medication with identical packaging (blister packs) and appearance (all drugs and placebo tablets were encapsulated). Subjects were required to return the blister packs to the investigator at the time of premature discontinuation or at the TOC visit for an assessment of compliance. The actual drug intake was documented in the case report form for each treatment day. Patients could receive other non-antimicrobial medication during the study and details of all concomitant drugs (particularly analgesics/NSAIDs) had to be recorded.

Table A Signs and scoring system for abdominal and pelvic tenderness. Adapted from McCormack *et al*[1]

- Direct and rebound abdominal tenderness in each of the four abdominal quadrants
- Cervical motion tenderness
- Uterine tenderness
- Right and left adnexal tenderness

Responses were scored as follows:
0 = tenderness absent
1+ = tenderness described by the patient but not manifested by changes in facial expression or muscle tone
2+ = tenderness resulting in altered facial expression or muscle tone
3+ = tenderness causing observable, marked distress
Total score was sum of the values. The maximum possible score was 36

Evaluable populations

The intent to treat (ITT)/safety population comprised those patients receiving at least one dose of study drug and undergoing at least one recorded observation thereafter. The per protocol (PP) population fulfilled all efficacy relevant protocol criteria, received medication for at least 72 hours (in cases of clinical failure) or 8 full days (in cases of clinical cure), were at least 80% compliant with medication and had a clinical evaluation at TOC (5–24 days post-therapy) that was not ''indeterminate.'' The microbiologically valid population (MBV) was a subset of the PP population who had at least one pre-therapy causative organism and a post-therapy bacteriological evaluation. In cervix/endocervix samples, only *N gonorrhoeae* and *C trachomatis* were considered causative, whereas all organisms (except coagulase negative staphylococci, diphteroids, *Corynebacterium* spp, and *Lactobacillus* spp) found in the endometrium samples were considered causative.

Efficacy evaluations

The primary efficacy outcome was clinical resolution at TOC (5–24 days post-therapy) in the PP population. Clinical resolution was defined as reduction of the pelvic pain score by >70% (McCormack score, table A)[1] plus apyrexia (rectal/tympanic/oral temperature value <38.0℃ or axillary/cutaneous temperature value <37.5℃) plus a white blood cell count $<10\ 500 \times 10^6$/l.

Secondary efficacy variables were: clinical response at follow up in the PP population (days 28–42 post-therapy), bacteriological response at TOC and follow up in the MBV population. Bacteriological success was defined as: ''eradication'' (absence of baseline causative organism on repeat culture without superinfection and/or negative PCR results at TOC) or ''presumed eradication'' (absence of repeat culture, but with an outcome of clinical resolution and invasive procedures not warranted).

Safety evaluations

Clinical adverse events and laboratory data were recorded for the ITT/safety population.

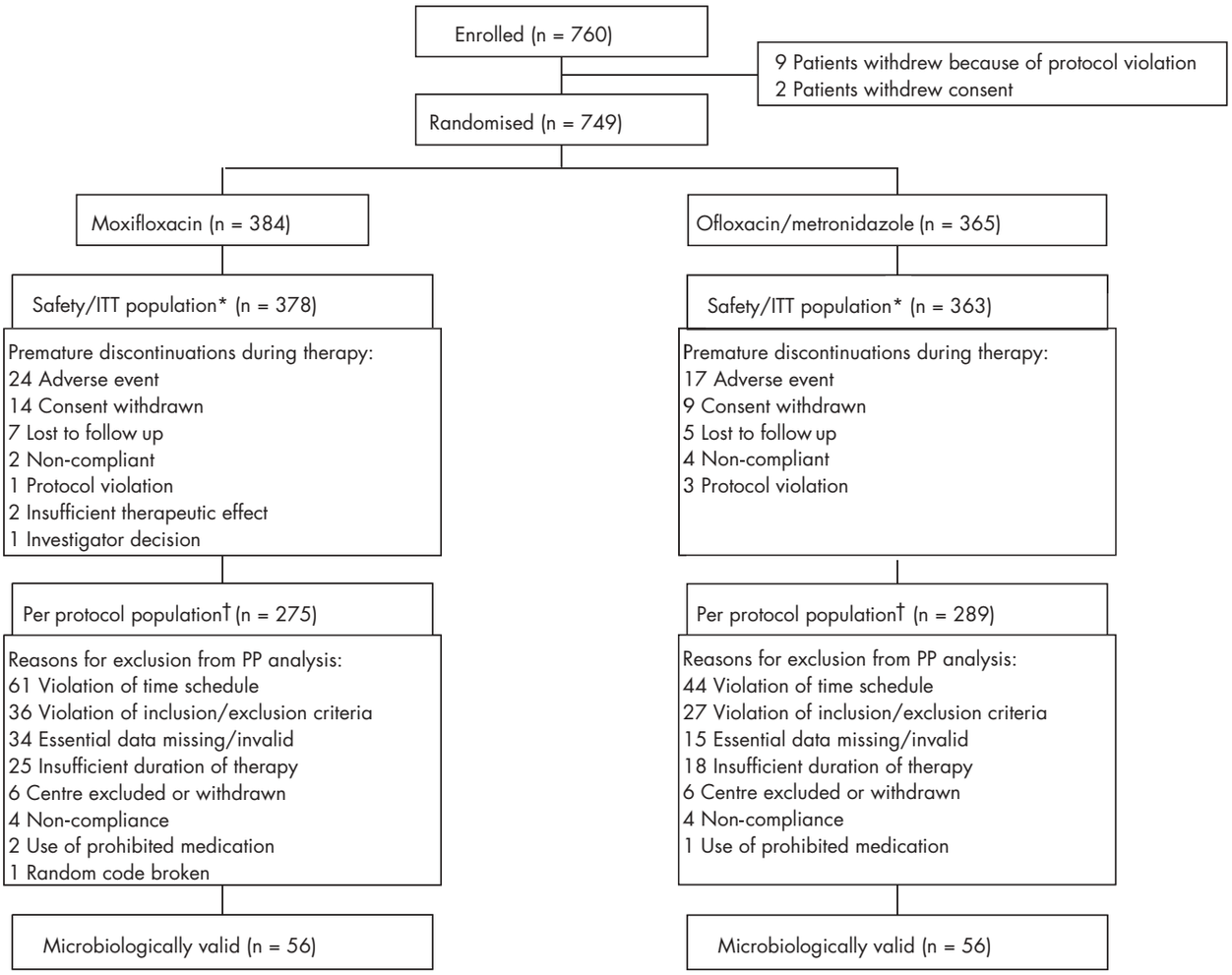
Statistical analysis

Demographic and baseline characteristics were summarised by treatment group and all treatment groups combined using the mean and standard deviation (SD), median, quartiles and minimum/maximum (quantitative data) or frequency counts (qualitative/categorical data). Treatment group comparability was checked for both the ITT and PP populations using two way analyses of variance (ANOVA), with respect to age, body mass index, duration of symptoms, and PID severity, and by a Cochran-Mantel-Haenszel test adjusted for centre/geographical region. The decision on how to combine centres was made before unblinding the study.

For the primary and secondary efficacy variables, a two sided 95% confidence interval (CI) for the difference (treatment group ''moxifloxacin'' minus ''comparator'') between clinical success rates of the two treatments was calculated using Mantel-Haenszel weighting. For moxifloxacin to be proved clinically ''not less effective'' than ofloxacin plus metronidazole, the lower limit of this CI had to be $>-10\%$ based on a target enrolment of 237 evaluable patients in each group. The trial was powered at 85% ($\alpha = 5\%$ (two sided)) based on the primary efficacy variable (clinical response at the TOC visit) resolution versus failure, and including a 10% adjustment to account for the multicentre study design. This yielded a sample size estimation of 237 patients per group. Assuming a validity rate of 75%, 316 patients were required for enrolment in each group.

REFERENCE

1 **McCormack WM**, Nowroozi K, Alpert S, *et al.* Acute pelvic inflammatory disease: characteristics of patients with gonococcal and nongonococcal infection and evaluation of their response to treatment with aqueous procaine penicillin G and spectinomycin hydrochloride. *Sex Transm Dis* 1977;**4**:125–31.



*6 moxifloxacin and 2 comparator randomised patients had no post-therapy evaluation
†Patients can have multiple reasons for exclusion from the PP analysis

Figure 1 Patient disposition and study flow. ITT, intent to treat; PP, per protocol.